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Patent Office

Ottawa, Canada
K1A 0C9

(21) (A1) 2,016,467
(22) 1990/05/10
(43) 1990/12/05
(52) 167-207

(51) INTL.CL.⁵ A61K-31/33

(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) **Method for Treating Peripheral Atherosclerotic Disease
Employing an HMG CoA Reductase Inhibitor and/or a
Squalene Synthetase Inhibitor**

(72) Eisman, Martin - U.S.A. ;

(73) Same as inventor

(30) (US) 361,520 1989/06/05

(57) 18 Claims

Notice: The specification contained herein as filed

Canada

ABSTRACTMETHOD FOR TREATING PERIPHERAL ATHEROSCLEROTIC
DISEASE EMPLOYING AN HMG CoA REDUCTASE
INHIBITOR AND/OR A SQUALENE SYNTHETASE INHIBITOR

A method is provided for treating peripheral atherosclerotic disease (arteriosclerosis obliterans) and/or intermittent claudication employing an HMG CoA reductase inhibitor alone and/or inhibitor of the enzyme squalene synthetase and optionally a pharmaceutical which reduces serum cholesterol by a mechanism other than inhibiting production of the enzyme HMG CoA reductase or the enzyme squalene synthetase, for example, probucol or gemfibrozil.

METHOD FOR TREATING PERIPHERAL ATHEROSCLEROTIC
DISEASE EMPLOYING AN HMG CoA REDUCTASE
INHIBITOR AND/OR A SQUALENE
SYNTHETASE INHIBITOR

5

The present invention relates to a method
for treating peripheral atherosclerotic disease
(arteriosclerosis obliterans) employing an inhibitor
10 of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA)
reductase or an inhibitor of squalene synthetase.

Harrison's Principles of Internal Medicine,
15 11th Edition, 1987, Editors-E. Braunwald et al,
Chapter 198, pp 1040-1046 by D. Eugene Strandness,
Jr., discusses vascular diseases of the
extremities - also referred to as peripheral
vascular disorders (Merck Manual, 15th Ed, Chapter
20 30, pp 555-557) - which include arteriosclerosis
obliterans. "Symptoms and signs that develop
secondary to arteriosclerosis obliterans rarely
have an abrupt onset, since the process is a
gradual, progressive one. The most common symptom
25 occurs with exercise and is termed intermittent
claudication, i.e., the pain that occurs in a
muscle(s) with an inadequate blood supply that is
stress d by exercis (page 1041)."

There are several different classes of compounds which have serum cholesterol lowering properties. Some of these compounds are inhibitors of the enzyme HMG CoA reductase which is essential in the production of cholesterol, such as
5 mevastatin (disclosed in U. S. Patent No. 3,983,140), lovastatin also referred to as mevinolin (disclosed in U. S. Patent No. 4,231,938), pravastatin (disclosed in U. S. Patent
10 No. 4,346,227) and velostatin also referred to as synvinolin (disclosed in U. S. Patents Nos. 4,448,784 and 4,450,171).

Other compounds which lower serum cholesterol may do so by an entirely different
15 mechanism than the HMG CoA reductase inhibitors. For example, serum cholesterol may be lowered through the use of bile acid sequestrants such as cholestyramine, colestipol, DEAE-Sephadex and poly(diallylmethylamine) derivatives (such as
20 disclosed in U. S. Patents Nos. 4,759,923 and 4,027,009) or through the use of antihyperlipoproteinemics such as probucol and gemfibrozil which apparently lower serum "low density lipoproteins" (LDL) and/or converts LDL into high
25 density lipoproteins (HDL).

U. S. Patent No. 4,759,923 mentioned above discloses that poly(diallylmethylamine) derivatives which are bile salt sequestrants may be used in conjunction with drugs which reduce serum
30 cholesterol by mechanisms other than sequestration, such as clofibrate, nicotinic acid, probucol, neomycin, p-aminosalicylic acid or mevinolin (also referred to as lovastatin).

Squalene synthetase is a microsomal enzyme which catalyzes the reductive dimerization of two molecules of farnesyl pyrophosphate (FPP) in the presence of nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) to form squalene (Poulter, C. D.; Rilling, H. C., in "Biosynthesis of Isoprenoid Compounds", Vol. I, Chapter 8, pp. 413-441, J. Wiley and Sons, 1981 and references therein). This enzyme is the first committed step of the de novo cholesterol biosynthetic pathway. The selective inhibition of this step should allow the essential pathways to isopentenyl tRNA, ubiquinone, and dolichol to proceed unimpeded. Squalene synthetase, along with HMG-CoA reductase has been shown to be down-regulated by receptor mediated LDL uptake (Faust, J. R.; Goldstein, J. L.; Brown, M. S. Proc. Nat. Acad. Sci. USA, 1979, 76, 5018-5022), lending credence to the proposal that inhibiting squalene synthetase will lead to an up-regulation of LDL receptor levels, as has been demonstrated for HMG-CoA reductase, and thus ultimately should be useful for the treatment and prevention of hypercholesterolemia and atherosclerosis.

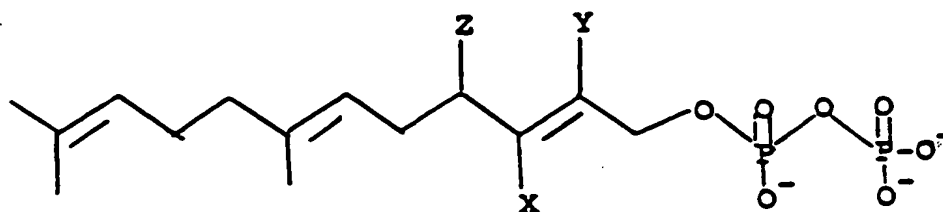
One approach to inhibitors of squalene synthetase is to design analogs of the substrate FPP. It is clear from the literature that the pyrophosphate moiety is essential for binding to the enzyme. However, such pyrophosphates are unsuitable as components of pharmacological agents due to their chemical and enzymatic lability towards allylic C-O cleavage, as well as their susceptibility to metabolism by phosphatases.

P. Ortiz de Montellano et al in
J. Med. Chem., 1977, 20, 243-249 describe the
 preparation of a series of substituted terpenoid
 pyrophosphates (Table A), and have shown these to
 5 be competitive inhibitors of the squalene
 synthetase enzyme. These substances retain the
 unstable allylic pyrophosphate moiety of FPP.

Table A

10

15



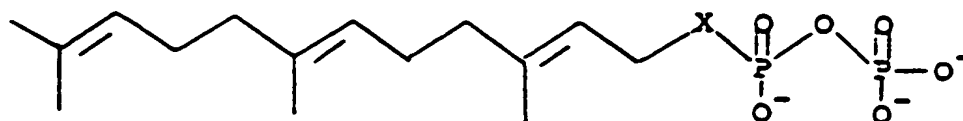
<u>No.</u>	<u>X</u>	<u>Y</u>	<u>Z</u>
1	CH ₃	CH ₃	H
2	H	H	H
20 3	C ₂ H ₅	H	H
4	I	H	H
5	H	I	H
6	CH ₃	H	SCH ₃

25

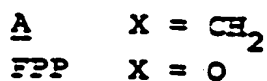
Corey and Volante, J. Am. Chem. Soc. 1976,
98, 1291-3, have prepared FPP analog A and
 presqualene pyrophosphate (PSQ-PP) analog B as
 inhibitors of squalene biosynthesis. (Presqualene
 pyrophosphate is an intermediate in the conversion
 30 of FPP to squalene). These inhibitors possess
 methylene groups in place of the allylic oxygen

moiety of FPP and PSQ-PP, but still retain the chemically and enzymatically unstable pyrophosphate linkage.

5

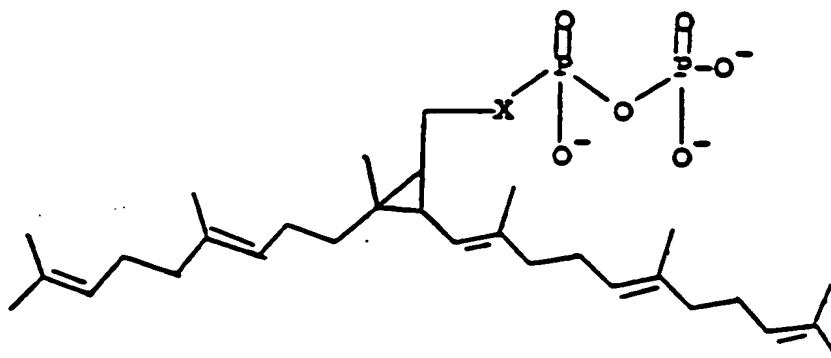


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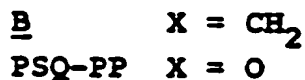


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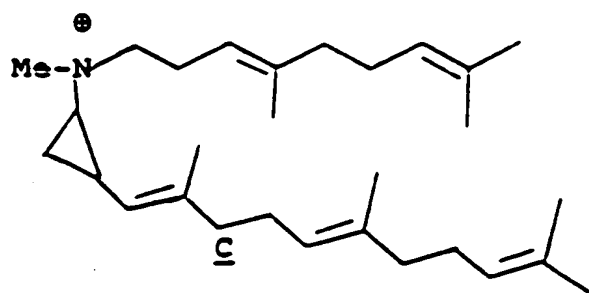


Poulter and co-workers have prepared cyclopropane C (Sandifer, R. M., et al., J. Am. Chem. Soc. 1982, 104, 7376-8) which in the

presence of inorganic pyrophosphate is an intermediate analog inhibitor of the enzyme squalene synthetase.

5

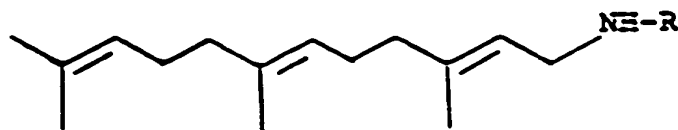
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Altman and co-workers, Bertolino, A., et al., Biochim. Biophys. Acta. 1978, 530, 17-23, reported that farnesyl amine and related derivatives D inhibit squalene synthetase, but provide evidence that this inhibition is non-specific and probably related to membrane disruption.

25

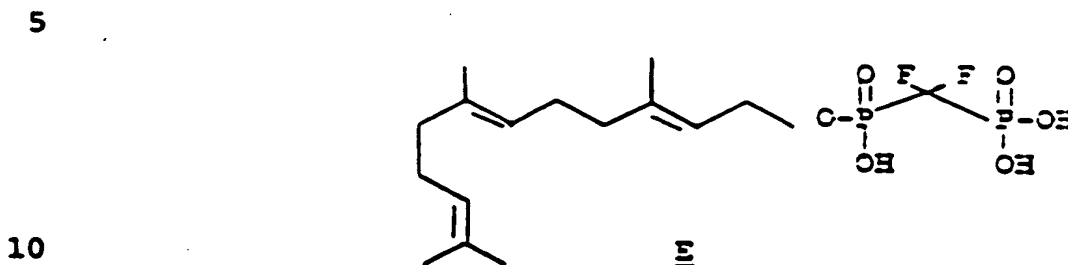


$R = H, CH_2CH_2OH, CH_2CH_2OCH_3$

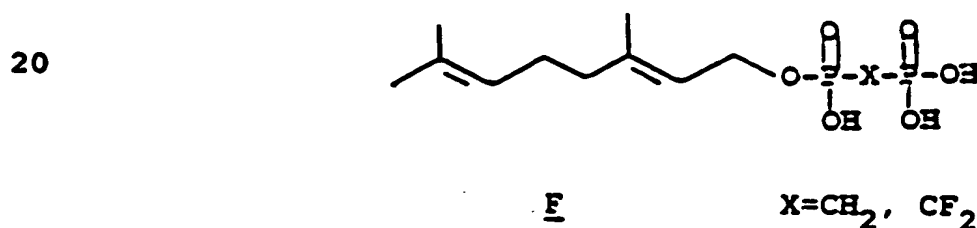
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D

Poulter, C.D., et al, J. Org. Chem., 1986, 51, 4768, prepared compound E in a demonstration of a synthetic method, but did not report any biological data.



Poulter, C.D., Stremmer, K.E., J.A.C.S., 1987, 109, 5542 describes the synthesis and biological evaluation of compounds having structure F. These compounds were evaluated as alternative substrates for avian liver farnesyl diphosphate and lemon peel cyclase.



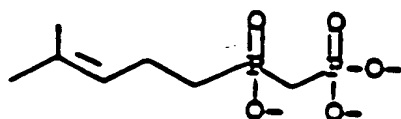
25 McClard, R. W. and Poulter, C. D., et al., J.A.C.S. 1987, 109, 5544, reported that phosphinylphosphonates G and H were competitive inhibitors of the 1'-4-condensation between isopentenyl diphosphate and geranyl diphosphate catalyzed by avian liver farnesyl diphosphate

30

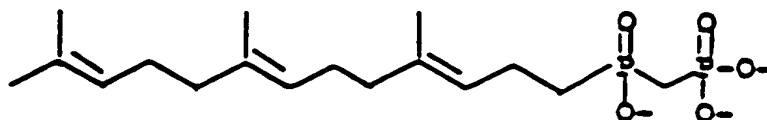
synthetase. Phosphinylphosphonates G and H had
Ki's of 19 μ M and 71 μ M, respectively. They also
reported the speculative isolation of the farnesyl
phosphinylphosphonate I, and the geranyl
5 phosphinylphosphonate J from the enzymatic reaction
of G with geranyl pyrophosphate or dimethylallyl
pyrophosphate, respectively. The structures of I
and J were tentatively assigned based on relative
TLC mobilities. They hypothesized that I could be
10 a potential inhibitor of squalene synthetase.



15

G

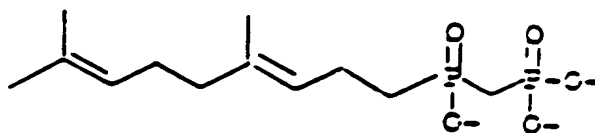
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H

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I

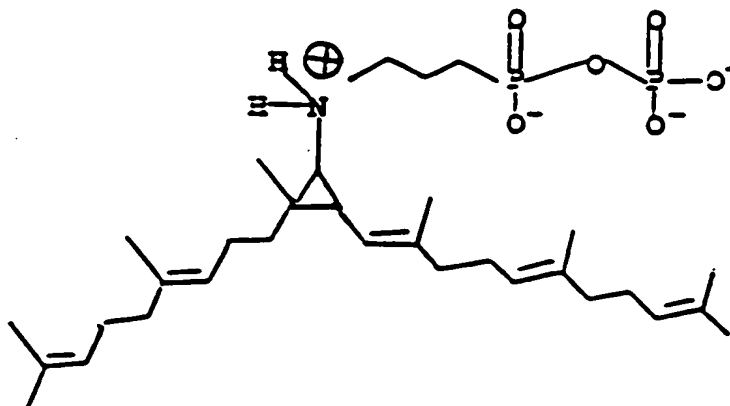
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J

Capson, T.L., PhD dissertation, June 1987,
 Dept. of Medicinal Chemistry, the University of
 10 Utah, Abstract, Table of Contents, pp. 16, 17,
 40-43, 48-51, Summary, discloses cyclopropanes of
 the structure discloses cyclopropanes of the
 structure

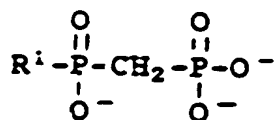
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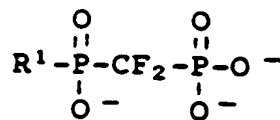


25 as intermediate analog inhibitors of squalene
 synthetase.

S. A. Biller et al., Journal of Medicinal
 Chemistry, 1988, Vol. 31, No. 10, pp 1869 to 1871
 disclose that isoprenoid (phosphinylmethyl)
 30 phosphonates (PMPs) inhibit squalene synthetase.
 These phosphonates have the structures



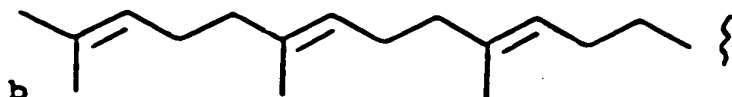
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2a-d3a,b

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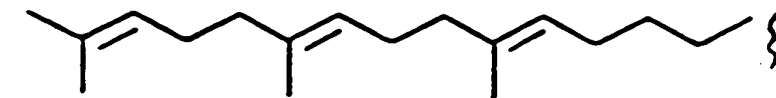
 R^1 

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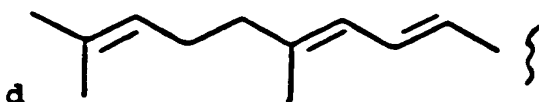


b

20



c



d

25

25 In accordance with the present invention, a
 method is provided for treating peripheral
 atherosclerotic disease also referred to as
 arteriosclerosis obliterans, and for treating
 30 intermittent claudication employing a cholesterol
 lowering drug which is an inhibitor of the enzyme
 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA)
 reductase or an inhibitor of the enzyme squalene

synthetase, a combination thereof or a combination of each with another pharmaceutical for lowering cholesterol, wherein a therapeutically effective amount of the above type compounds is systemically, such as orally or parenterally, administered over a prolonged period.

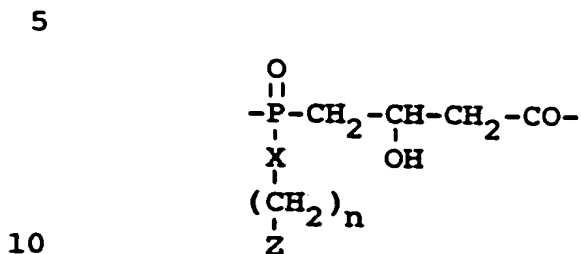
Where the combination of the HMG CoA reductase inhibitor and squalene synthetase inhibitor are employed, the HMG CoA reductase inhibitor will be employed in a weight ratio to the squalene synthetase inhibitor of within the range of from about 0.001:1 to about 1000:1 and preferably from about 0.05:1 to about 100:1.

The pharmaceutical (also referred to as other serum cholesterol lowering agent) reduces serum cholesterol and/or inhibits cholesterol biosynthesis by a mechanism other than by inhibiting production of the enzyme squalene synthetase or 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG CoA) reductase, such as a bile salt sequestrant or antihyperlipoproteinemic agent which inhibits formation of LDL or converts LDL to HDL. The HMG CoA reductase inhibitor or squalene synthetase inhibitor will be employed in a weight ratio to the "pharmaceutical" of within the range of from about 0.001:1 to about 1000:1 and preferably from about 0.05:1 to about 100:1.

Cholesterol lowering drugs or drugs which are inhibitors of cholesterol biosynthesis which may be used in the method of the invention include HMG CoA reductase inhibitors or squalene synthetase inhibitors.

The HMG CoA reductase inhibitors suitable for use herein include, but are not limited to, mevastatin and related compounds as disclosed in U. S. Patent No. 3,983,140, lovastatin (mevinolin) and related compounds as disclosed in U. S. Patent No. 4,231,938, pravastatin and related compounds such as disclosed in U. S. Patent No. 4,346,227, velostatin (synvinolin) and related compounds as disclosed in U. S. Patents Nos. 4,448,784 and 4,450,171, with lovastatin, pravastatin or velostatin being preferred. Other HMG CoA reductase inhibitors which may be employed herein include, but are not limited to, fluindostatin (Sandoz XU-62-320), pyrazole analogs of mevalonolactone derivatives as disclosed in U. S. Patent No. 4,613,610, indene analogs of mevalonolactone derivatives as disclosed in PCT application WO 86/03488, 6-[2-(substituted-pyrrol-1-yl)alkyl]-pyran-2-ones and derivatives thereof as disclosed in U. S. Patent No. 4,647,576, Searle's SC-45355 (a 3-substituted pentanedioic acid derivative) dichloroacetate, imidazole analogs of mevalonolactone as disclosed in PCT application WO 86/07054, 3-carboxy-2-hydroxy-propane-phosphonic acid derivatives as disclosed in French Patent No. 2,596,393, 2,3-di-substituted pyrrole, furan and thiophene derivatives as disclosed in European Patent Application No. 0221025, naphthyl analogs of mevalonolactone as disclosed in U. S. Patent No. 4,686,237, octahydro-naphthalenes such as disclosed in U. S. Patent No. 4,499,289, keto analogs of mevinolin (lovastatin) as disclosed in European Patent Application No. 0,142,146 A2, as well as other known HMG CoA reductase inhibitors.

In addition, compounds useful in inhibiting HMG CoA reductase suitable for use herein are disclosed in GB 2205837 which compounds have the moiety

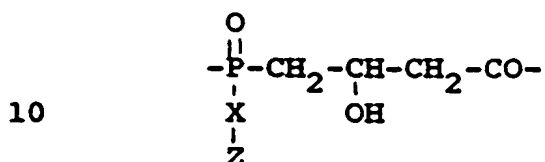


wherein X is -O- or -NH-, n is 1 or 2 and Z is a hydrophobic anchor.

Examples of such compounds include (S)-4-
 15 [[[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]-methoxy]methoxyphosphinyl]-3-hydroxy-butanoic acid, methyl ester or its monolithium salt,
 (S)-4-[[[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]methoxy]hydroxyphosphinyl]-3-
 20 hydroxybutanoic acid, dilithium salt,
 (3S)-4-[[[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]methoxy]methylphosphinyl]-3-hydroxybutanoic acid, monolithium salt,
 (S)-4-[[[2,4-dichloro-6-[(4-fluorophenyl)-methoxy]phenyl]methoxy]methoxyphosphinyl]-3-
 25 hydroxybutanoic acid, monolithium salt,
 (3S)-4-[[[2,4-dichloro-6-[(4-fluorophenyl)-methoxy]phenyl]methoxy]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt,
 30 (3S)-4-[[[2,4-dichloro-6-[(4-fluorophenyl)-methoxy]phenyl]methoxy]methylphosphinyl]-3-hydroxybutanoic acid, or its methyl ester, and

(S)-4-[[[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl-2-yl]methyl]amino]methoxyphosphinyl]-3-hydroxybutanoic acid, monolithium salt.

Another class of HMG CoA reductase inhibitors suitable for use herein include compounds disclosed in GB 2205838, which compounds have the moiety



wherein X is $\text{-CH}_2\text{-}$, $\text{-CH}_2\text{-CH}_2\text{-}$, -CH=CH- , $\text{-CH}_2\text{CH}_2\text{CH}_2\text{-}$, $\text{-C}\equiv\text{C-}$ or $\text{-CH}_2\text{O-}$, where O is linked to Z, and Z is a hydrophobic anchor.

Examples of such compounds include (S)-4-[[[(E)-2-[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt;

(S)-4-[[2-[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, methyl ester or mono- or di-alkali metal salts thereof;

(S)-4-[[[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic acid or the methyl ester thereof;

(5Z)-4-[[2-[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, methyl esters thereof;

(S)-4-[[2-[3-(4-fluorophenyl)-1-(1-methyl-ethyl)-1H-indol-2-yl]ethyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl esters;

(S)-4-[[2-[[1,1'-biphenyl]-2-yl]ethyl]-methoxyphosphinyl-3-hydroxybutanoic acid, methyl ester;

(S)-4-[[2-[4'-fluoro-3,3',5-trimethyl-[1,1'-biphenyl]-2-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(S)-4-[[2-[4'-fluoro-3,3',5-trimethyl-[1,1'-biphenyl]-2-yl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(SZ)-4-[[2-[4'-fluoro-3,3',5-trimethyl-[1,1'-biphenyl]-2-yl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(S)-4-[[2-[3-(4-fluorophenyl)-1-(1-methyl-ethyl)-1H-indol-2-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(S)-4-[[2-[(1,1'-biphenyl)-2-yl]ethyl]-hydroxyphosphinyl]-3-butanoic acid, dilithium salt;

(S)-4-(hydroxymethoxyphosphinyl)-3-[[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic acid, methyl ester, or its dicyclohexylamine (1:1) salt;

(S)-4-[[2-[1-(4-fluorophenyl)-3-(1-methyl-ethyl)-1-indol-2-yl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

(S)-4-[[2-[1-(4-fluorophenyl)-3-(1-methyl-ethyl)-1H-indol-2-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

(E)-4-[[2-[3-(4-fluorophenyl)-1-(1-methyl-ethyl)-1H-indol-2-yl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

5 4-[[2-[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

10 (E)-4-[[2-[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

15 (S)-4-[[[2,4-dimethyl-6-[(4-fluorophenyl)-methoxy]phenyl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

20 (S)-4-[[[2,4-dimethyl-6-[(4-fluorophenyl)-methoxy]phenyl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

(S)-4-[[2-[3,5-dimethyl[1,1'-biphenyl]-2-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

25 (S)-4-[[2-[4'-fluoro-3,5-dimethyl[1,1'-biphenyl]-2-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

30 (S)-4-[[2-[[1,1'-biphenyl]-2-yl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

(S)-4-[[2-(5-(4-fluorophenyl)-3-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;

- (S)-4-[[2-[5-(4-fluorophenyl)-3-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;
- 5 (E)-4-[[2-[5-(4-fluorophenyl)-3-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethenyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;
- (E)-4-[[2-[5-(4-fluorophenyl)-3-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;
- 10 (S)-4-[[2-[5-(4-fluorophenyl)-3-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;
- (S)-4-[[2-[5-(4-fluorophenyl)-3-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;
- 15 (S)-4-[[2-[3-(4-fluorophenyl)-5-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;
- (S)-4-[[2-[3-(4-fluorophenyl)-5-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;
- 20 (S)-4-[[2-[3-(4-fluorophenyl)-5-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;
- 25 (S)-4-[[2-[3-(4-fluorophenyl)-5-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;
- (S)-4-[[[4-(4-fluorophenyl)-1-(1-methyl-ethyl)-3-phenyl-1H-pyrazol-5-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;
- 30 (S)-4-[[[4-(4-fluorophenyl)-1-(1-methyl-ethyl)-3-phenyl-1H-pyrazol-5-yl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

- (S)-4-[[2-[4-(4-fluorophenyl)-1-(1-methyl-ethyl)-3-phenyl-1H-pyrazol-5-yl]ethyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;
- 5 (S)-4-[[2-[4-(4-fluorophenyl)-1-(1-methyl-ethyl)-3-phenyl-1H-pyrazol-5-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;
- (S)-4-[[[1-(4-fluorophenyl)-4-(1-methyl-ethyl)-2-phenyl-1H-imidazol-5-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;
- 10 (S)-4-[[[1-(4-fluorophenyl)-4-(1-methyl-ethyl)-2-phenyl-1H-imidazol-5-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;
- (S)-4-[[2-[1-(4-fluorophenyl)-4-(1-methyl-ethyl)-2-phenyl-1H-imidazol-5-yl]ethyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;
- 15 (S)-4-[[2-[1-(4-fluorophenyl)-4-(1-methyl-ethyl)-2-phenyl-1H-imidazol-5-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;
- (S)-4-[[[2-(cyclohexylmethyl)-4,6-dimethyl-phenyl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;
- 20 4-[[2-[2-(cyclohexylmethyl)-4,6-dimethyl-phenyl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;
- 25 (S)-4-[[2-[2-(cyclohexylmethyl)-4,6-dimethyl-phenyl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;
- 30 4-[[[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]oxy]methyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

4-[[[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]methyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

5 (S)-4-[[[1-(4-fluorophenyl)-3-methyl-2-naphthalenyl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

10 (E)-4-[[2-[1-(4-fluorophenyl)-3-methyl-2-naphthalenyl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

15 (S)-4-[[2-[1-(4-fluorophenyl)-3-methyl-2-naphthalenyl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

4-[[3-[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]propyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;

20 4-[[3-[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]propyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

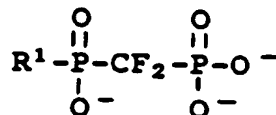
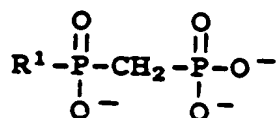
25 [1S-[1<a(R*),2<a,4a<b,8<b,8a<a]]-4-[[2-[8-(2,2-dimethyl-1-oxobutoxy)decahydro-2-methyl-1-naphthalenyl]ethyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;

30 [1S-[1<a(R*),2<a,4a<b,8<b,8a<a]]-4-[[2-[8-(2,2-dimethyl-1-oxobutoxy)decahydro-2-methyl-1-naphthalenyl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

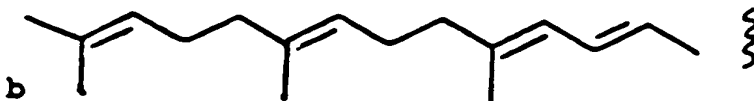
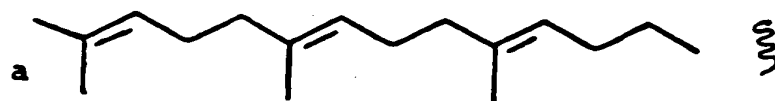
(S)-4-[[[3'-(4-fluorophenyl)spiro]cyclopentan-1,1'-[1H]indene]-2-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester; and

(S)-4-[[[3'-(4-fluorophenyl)spiro]cyclopentane-1,1'-[1H]indene]-2-yl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt.

The squalene synthetase inhibitors suitable for use herein include, but are not limited to, those disclosed by Biller et al., supra, including isoprenoid (phosphinylmethyl)phosphonates such as those of the formula

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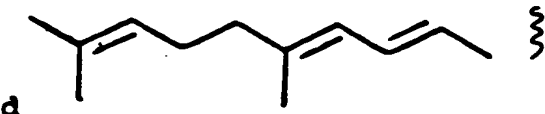
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including the triacids thereof, triesters thereof and tripotassium and trisodium salts thereof as well as other squalene synthetase inhibitors disclosed in pending U.S. patent application Serial
5 No. 141,744, filed January 11, 1988 and in Biller et al, J. Med. Chem., 1988, Vol. 31, No. 10, pp 1869 to 1871.

In addition, other squalene synthetase inhibitors suitable for use herein include the
10 terpenoid pyrophosphates disclosed by P. Ortiz de Montellano et al., J. Med. Chem.; 1977, 20, 243-249, the farnesyl diphosphate analog A and presqualene pyrophosphate (PSQ-PP) analogs as disclosed by Corey and Volante, J. Am. Chem. Soc.
15 1976, 98, 1291-1293, phosphinylphosphonates reported by McClard, R. W. et al., J.A.C.S., 1987, 109, 5544 and cyclopropanes reported by Capson, T.L., PhD dissertation, June, 1987, Dept. Med. Chem. U. of Utah, Abstract, Table of Contents,
20 pp. 16, 17, 40-43, 48-51, Summary.

The disclosure of the above-mentioned patents and patent applications are incorporated herein by reference.

Preferred are pravastatin, lovastatin or
25 velostatin or a squalene synthetase inhibitor such as disclosed by Biller et al., supra or combinations thereof which include a weight ratio of the HMG CoA reductase inhibitor:squalene synthetase inhibitor of from about 0.05:1 to about 100:1.

The "pharmaceutical" or other serum cholesterol lowering agents which function other than by inhibiting the enzyme HMG CoA reductase or squalene synthetase suitable for use herein include, but are not limited to, antihyperlipoproteinemic agents such as probucol, and gemfibrozil and related compounds as disclosed in U. S. Patent No. 3,674,836, probucol and gemfibrozil being preferred, bile acid sequestrants such as cholestyramine, colestipol and DEAE-Sephadex (Secholex[®], Polidexide[®]), as well as clofibrate, lipostabil (Rhone-Poulenc), Eisai E-5050 (an N-substituted ethanolamine derivative), imanixil (HOE-402) tetrahydrolipstatin (THL), istigmastanyl-phosphorylcholine (SPC, Roche), aminocyclodextrin (Tanabe Seiyoku), Ajinomoto AJ-814 (azulene derivative), melinamide (Sumitomo), Sandoz 58-035, American Cyanamid CL-277,082 and CL-283,546 (di-substituted urea derivatives), nicotinic acid, neomycin, p-aminosalicylic acid, aspirin, poly(diallylmethylamine) derivatives such as disclosed in U. S. Patent No. 4,759,923, quaternary amine poly(diallyldimethylammonium chloride) and ionenes such as disclosed in U. S. Patent No. 4,027,009, and other known serum cholesterol lowering agents which lower cholesterol through a mechanism other than by the inhibition of the enzyme HMG CoA reductase or squalene synthetase.

Also preferred are combinations of any of the HMG CoA reductase inhibitors or isoprenoid (phosphinylmethyl) phosphonates disclosed by Biller et al., supra with probucol or gemfibrozil.

In carrying out the method of the present invention, the cholesterol lowering drug may be administered to mammalian species, such as monkeys, dogs, cats, rats, humans, etc. and as such may be incorporated in a conventional systemic dosage form, such as a tablet, capsule, elixir or injectable. The above dosage forms will also include the necessary carrier material, excipient, lubricant, buffer, antibacterial, bulking agent (such as mannitol), anti-oxidants (ascorbic acid of sodium bisulfite) or the like. Oral dosage forms are preferred, although parenteral forms are quite satisfactory as well.

The dose administered must be carefully adjusted according to age, weight and condition of the patient, as well as the route of administration, dosage form and regimen and the desired result.

Thus, for oral administration, a satisfactory result may be obtained employing the HMG CoA reductase inhibitor in dosages employed, for example, for lovastatin as indicated in the Physician's Desk Reference, such as in an amount within the range of from about 1 to 2000 mg, and preferably from about 4 to about 200 mg in. The squalene synthetase inhibitor may be employed in dosages in an amount within the range of from about 10 mg to about 2000 mg and preferably from about 25 mg to about 200 mg. The HMG CoA reductase inhibitor and squalene synthetase inhibitor may each be employed alone or may be employed together in the same oral dosage form or in separate oral dosage forms taken at the same time.

A preferred oral dosage form, such as tablets or capsules, will contain the HMG CoA reductase inhibitor in an amount of from about 0.1 to about 100 mg, preferably from about 5 to about 80 mg, and more preferably from about 10 to about 40 mg.

A preferred oral dosage form, such as tablets or capsules will contain the squalene synthetase inhibitor in an amount of from about 10 to about 500 mg, preferably from about 25 to about 200 mg.

The other serum cholesterol lowering agent when present will be employed in dosages normally employed as indicated in the Physician's Desk Reference, for each of such agents such as in an amount within the range of from about 2 mg to about 7500 mg and preferably from about 2 mg to about 4000 mg with the HMG CoA reductase inhibitor or squalene synthetase inhibitor and other serum cholesterol lowering agent being employed together in the same oral dosage form or in separate oral dosage forms taken at the same time.

The compositions described above may be administered in the dosage forms as described above in single or divided doses of one to four times daily. It may be advisable to start a patient on a low dose combination and work up gradually to a high dose combination.

Tablets of various sizes can be prepared, e.g., of about 2 to 2000 mg in total weight, containing one or both of the active substances in the ranges described above, with the remainder

being a physiologically acceptable carrier of other materials according to accepted pharmaceutical practice. These tablets can, of course, be scored to provide for fractional doses. Gelatin capsules
5 can be similarly formulated.

Liquid formulations can also be prepared by dissolving or suspending one or the combination of active substances in a conventional liquid vehicle acceptable for pharmaceutical administration so as
10 to provide the desired dosage in one to four teaspoonsful.

Such dosage forms can be administered to the patient on a regimen of one to four doses per day.

According to another modification, in order
15 to more finely regulate the dosage schedule, the active substances may be administered separately in individual dosage units at the same time or carefully coordinated times. Since blood levels are built up and maintained by a regulated schedule
20 of administration, the same result is achieved by the simultaneous presence of the two substances. The respective substances can be individually formulated in separate unit dosage forms in a , manner similar to that described above.

25 Where combinations are desired, fixed combinations of HMG CoA reductase inhibitor and squalene synthetase inhibitor are and/or other cholesterol lowering agents are more convenient and are preferred, especially in tablet or capsule form
30 for oral administration.

In formulating the compositions, the active substances, in the amounts described above, are compounded according to accepted pharmaceutical practice with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., in the particular type of unit dosage form.

Illustrative of the adjuvants which may be incorporated in tablets are the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate or cellulose; a disintegrating agent such as corn starch, potato starch, alginic acid or the like; a lubricant such as stearic acid or magnesium stearate; a sweetening agent such as sucrose, aspartame, lactose or saccharin; a flavoring agent such as orange, peppermint, oil of wintergreen or cherry. When the dosage unit form is a capsule, it may contain in addition to materials of the above type a liquid carrier such as a fatty oil. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets or capsules may be coated with shellac, sugar or both. A syrup of elixir may contain the active compound, water, alcohol or the like as the carrier, glycerol as solubilizer, sucrose as sweetening agent, methyl and propyl parabens as preservatives, a dye and a flavoring such as cherry or orange.

Some of the active substances described above form commonly known, pharmaceutically acceptable salts such as alkali metal and other

common basic salts or acid addition salts, etc. References to the base substances are therefore intended to include those common salts known to be substantially equivalent to the parent compound.

- 5 The formulations as described above will be administered for a prolonged period, that is, for as long as the potential for arteriosclerosis obliterans and intermittent claudication remains or the symptoms continue. Sustained release forms of
- 10 such formulations which may provide such amounts biweekly, weekly, monthly and the like may also be employed. A dosing period of at least one to two weeks are required to achieve minimal benefit.

The following Examples represent preferred embodiments of the present invention. All temperatures are expressed in degrees Centigrade unless otherwise indicated and all mesh sizes are U.S. Standard ASTME.

Example 1

A pravastatin formulation in the form of tablets having the following composition was prepared as described below.

	<u>Ingredient</u>	<u>Parts by Weight</u>
	Pravastatin	7
	Lactose	67
15	Microcrystalline cellulose	20
	Croscarmellose sodium	2
	Magnesium stearate	1
	Magnesium oxide	3

Pravastatin, magnesium oxide and a fraction (30%) of the lactose were mixed together for 2 to 10 minutes employing a suitable mixer. The resulting mixture was passed through a #12 to #40 mesh size screen. Microcrystalline cellulose, croscarmellose sodium and the remaining lactose were added and the mixture was mixed for 2 to 10 minutes. Thereafter, magnesium stearate was added and mixing was continued for 1 to 3 minutes.

The resulting homogeneous mixture was then compressed into tablets each containing 5 mg, 10 mg, 20 or 40 mg pravastatin which may be used to treat peripheral vascular disease (arteriosclerosis obliterans) and intermittent claudication.

Example 2

Tablets of the following compositions are prepared as described below.

	<u>Ingredient</u>	<u>Weight (mg)</u>
5	(E,E)-[difluoro[hydroxy(4,8,12-trimethyl-3,7,11-tridecatrienyl)-phosphinyl]methyl]phosphonic acid tripotassium salt (squalene synthetase inhibitor prepared as described by Biller et al. supra)	100 mg
10	Avicel	112.5 mg
	Lactose	113 mg
	Cornstarch	17.5 mg
15	Stearic Acid	<u>7 mg</u>
		350 mg

The tablets are prepared from sufficient bulk quantities by slugging the squalene synthetase inhibitor Avicel, and a portion of the stearic acid. The slugs are ground and passed through a #2 screen and then mixed with the lactose, cornstarch, and the remainder of stearic acid. The mixture is compressed into 350 mg capsule shaped tablets in a tablet press. The tablets are scored for dividing in half.

The squalene synthetase inhibitor tablets may be administered as in accordance with the teachings of the present invention to treat arteriosclerosis obliterans and/or intermittent claudication. In addition, the pravastatin and squalene synthetase inhibitor tablets of Example 1 and 2 may be ground up into powders and used together in a single capsule.

Example 3

A pravastatin formulation in the form of tablets, each containing 5 mg, 10 mg, 20 mg or 40 mg pravastatin, having the following composition was prepared as described in Example 1, except that color was incorporated into the powder mixture containing pravastatin, magnesium oxide and a fraction of the lactose.

10	<u>Ingredient</u>	<u>Parts by Weight</u>
	Pravastatin	7
	Lactose	67
	Microcrystalline cellulose	20
	Croscarmellose sodium	2
15	Magnesium stearate	1
	Magnesium oxide	3
	FD&C Red #3 Lake	0.2

The above pravastatin tablet may be administered to treat arteriosclerosis obliterans and/or intermittent claudication with or without a squalene synthetase inhibitor tablet (described in Example 2) in accordance with the teachings of the present invention.

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Examples 4 and 5

Lovastatin tablets are prepared employing conventional pharmaceutical techniques containing 20 mg lovastatin, cellulose, color, lactose, magnesium stearate and starch and butylated hydroxyanisole as a preservative as described in the 1988 PDR.

30

The lovastatin tablets may be employed alone or in combination with the squalene synthetase inhibitor tablets (described in Example 2) in separate or combined dosage forms to treat arterio-sclerosis obliterans or intermittent claudication in accordance with the present invention.

Examples 6 to 8

A formulation in the form of tablets having the following composition is prepared as described in Example 1.

<u>Ingredient</u>	<u>Weight (mg)</u>
(E,E,E)-[difluoro[hydroxy(4,8,12-trimethyl-1,3,7,11-tridecate- traenyl)phosphinyl)methyl]- phosphonic acid tripotassium salt (squalene synthetase inhibitor prepared as described by Biller et al. supra)	100 mg
Cornstarch	50 mg
Gelatin	7.5 mg
Avicel (microcrystalline cellulose)	25 mg
Magnesium stearate	<u>2.5 mg</u>
	185 mg

The above formulations alone or with pravastatin tablets, or lovastatin tablets described in Examples 1 and 4, respectively, or velostatin tablets may be employed in separate dosage forms or combined in a single capsule form to treat arterio-sclerosis obliterans and/or intermittent claudication in accordance with the present invention.

Example 9

Probuco1 tablets containing 250 mg probuco1 are prepared employing conventional procedures containing the following additional ingredients as
5 set out in the 1988 PDR: corn starch, ethyl cellulose, glycerin, hydroxypropyl cellulose, hydroxypropylmethyl cellulose 2910, iron oxide, lactose, magnesium stearate, microcrystalline cellulose, polysorbate 80, talc and titanium
10 dioxide.

The squalene synthetase inhibitor formulations or HMG CoA reductase inhibitor formulations described in the previous examples may be employed with probuco1 tablets as a combination in accordance
15 with the teachings of the present invention to treat arteriosclerosis obliterans and/or intermittent claudication. In addition, any or all of the above drugs and probuco1 tablets may be ground up into powders and used together in a single capsule.

20

Example 10

Capsules containing 300 mg gemfibrozil are prepared employing conventional pharmaceutical techniques containing the following additional
25 ingredients as described in the 1988 PDR: polysorbate 80 NF, starch NF and silica gel.

The squalene synthetase inhibitor tablets or HMG CoA reductase inhibitor tablets previously described and gemfibrozil capsules may be adminis-
30 tered as a combination or the HMG CoA reductase inhibitor or squalene synthetase inhibitor tablet may be ground into a powder and used in a single

capsule containing gemfibrozil to treat arterio-sclerosis obliterans and/or intermittent claudication.

5

Examples 11

The above described HMG CoA reductase inhibitor tablets or squalene synthetase inhibitor tablets may be employed in combination with clofibrate capsules containing 500 mg clofibrate and inactive ingredients including color, and gelatin as described in the 1988 PDR to treat arteriosclerosis obliterans and/or intermittent claudication in accordance with the present invention.

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Examples 12

Squalene synthetase inhibitor tablets or HMG CoA reductase inhibitor tablets as described above may be employed in combination with cholestyramine resin containing 4 g cholestyramine, acacia, citric acid, color, flavor, polysorbate 80, propylene glycol alginate and sucrose as described in the 1988 PDR to treat arteriosclerosis obliterans and/or intermittent claudication in accordance with the present invention.

20
25Examples 13

Squalene synthetase inhibitor tablets, or HMG CoA reductase inhibitor tablets described above may be employed in combination with nicotinic acid, colestipol, dextrothyroxine or other serum cholesterol lowering agent in

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accordance with the teaching of the present
invention to treat arteriosclerosis obliterans
and/or intermittent claudication.

It will also be appreciated that any of the
5 HMG CoA reductase inhibitors or any of the squalene
synthetase inhibitors disclosed herein may be
employed in combination with each other and/or with
any of the serum cholesterol lowering agents
disclosed herein in accordance with the present
10 invention.

What we claim is:

1. A method for treating arteriosclerosis obliterans and/or intermittent claudication, which comprises administering to a mammalian species in need of treatment a therapeutically effective amount of an inhibitor of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase or an inhibitor of the enzyme squalene synthetase or combinations thereof.

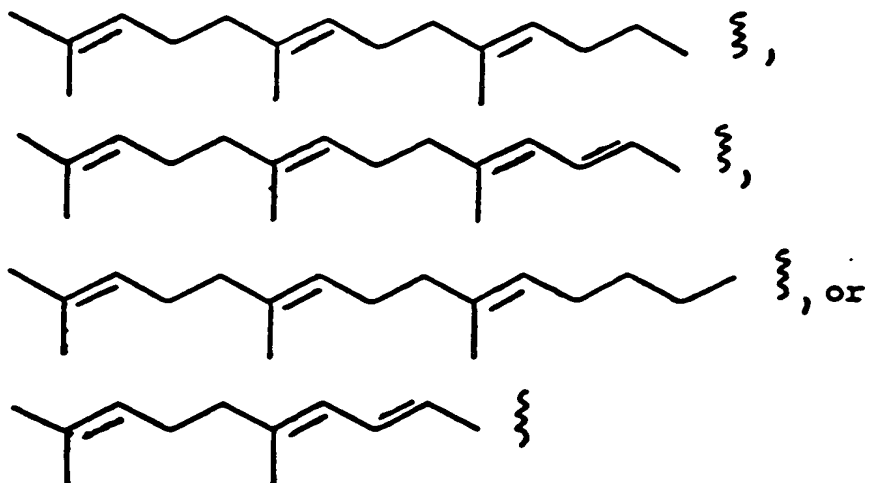
2. The method as defined in Claim 1 wherein said inhibitor of the enzyme HMG CoA reductase is mevastatin, lovastatin, pravastatin or velostatin.

3. The method as defined in Claim 1 wherein said inhibitor of the enzyme HMG CoA reductase is a pyrazole analog of a mevalonolactone, an indene analog of mevalonolactone, a 3-carboxy-2-hydroxy-propane-phosphonic acid derivative, a 6-[2-(substituted-pyrrol-1-yl)-alkyl]pyran-2-one, an imidazole analog of mevalonolactone, or a heterocyclic analog of mevalonolactone, a naphthyl analog of mevalonolactone, an octahydro-naphthalene, fluindostatin, a keto analog of lovastatin or a 2,3-di-substituted pyrrole, furan or thiophene.

4. The combination as defined in Claim 1 wherein the inhibitor of the enzyme squalene synthetase has the formula



wherein R¹ is



5. The method as defined in Claim 1 wherein the inhibitor of the enzyme HMG CoA reductase is present in a weight ratio to said inhibitor of the enzyme squalene synthetase of within the range of from about 0.001:1 to about 1000:1.

6. The method as defined in Claim 5 wherein the inhibitor of the enzyme HMG CoA reductase is lovastatin, pravastatin or velostatin.

7. The method as defined in Claim 1 wherein the inhibitor of the enzyme squalene synthetase is administered alone.

8. The method as defined in Claim 1 wherein the inhibitor of the enzyme HMG CoA reductase is lovastatin, pravastatin or velostatin is administered above.

9. The method as defined in Claim 1 where the inhibitor of the enzyme squalene synthetase or the HMG CoA reductase inhibitor is administered in combination with a pharmaceutical which reduces serum cholesterol and/or inhibits cholesterol biosynthesis by a mechanism other than inhibiting production of the enzyme HMG CoA reductase or squalene synthetase.

10. The method as defined in Claim 9 wherein said pharmaceutical is probucol, gemfibrozil, clofibrate, dextrothyroxine or its sodium salt, colestipol or its hydrochloride, cholestyramine, nicotinic acid, neomycin, p-aminosalicylic acid or aspirin.

11. The method as defined in Claim 10 wherein said pharmaceutical is a bile acid sequestrant.

12. The method as defined in Claim 11 wherein said bile acid sequestrant is cholestyramine, colestipol, DEAE-Sephadex, a poly(diallylmethylamine) derivative, an ionene or the quaternary amine poly(diallyldimethylammonium) chloride.

13. The method as defined in Claim 10 wherein said pharmaceutical is probucol or gemfibrozil.

14. The method as defined in Claim 9 wherein said pharmaceutical is probucol, gemfibrozil, nicotinic acid, cholestyramine,

clofibrate, colestipol or p-aminosalicylic acid and the HMG CoA reductase inhibitor is pravastatin, lovastatin or velostatin.

15. A method for treating intermittent claudication, which comprises administering to a mammalian species in need of treatment a therapeutically effective amount of an HMG CoA reductase inhibitor or a squalene synthetase inhibitor or a combination thereof.

16. The method as defined in Claim 15 wherein the HMG CoA reductase inhibitor is pravastatin, lovastatin or velostatin.

17. A method for treating arteriosclerosis obliterans, which comprises administering to a mammalian species in need of treatment a therapeutically effective amount of an HMG CoA reductase inhibitor or a squalene synthetase inhibitor or a combination thereof.

18. The method as defined in Claim 17 wherein the HMG CoA reductase inhibitor is pravastatin, lovastatin or velostatin.